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(54) Title: PLANT DEVELOPMENTAL GENES

(57) Abstract: Recombinant polynucleotides and methods for modifying the phenotype of a plant are provided. In particular, the phenotype that is being modified is a plant's structure and development characteristics.

<b>PLANT DEVELOPMENTAL GENES</b>
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**RELATED APPLICATION INFORMATION**

The present invention claims the benefit from US Provisional Patent Application Serial  
5 Nos. 60/166,228 filed November 17, 1999 and 60/197,899 filed April 17, 2000 and "Plant Trait  
Modification III" filed August 22, 2000.

**FIELD OF THE INVENTION**

This invention relates to the field of plant biology. More particularly, the present  
invention pertains to compositions and methods for phenotypically modifying a plant.

10

**BACKGROUND OF THE INVENTION**

Transcription factors can modulate gene expression, either increasing or  
decreasing (inducing or repressing) the rate of transcription. This modulation results in  
differential levels of gene expression at various developmental stages, in different tissues and cell  
types, and in response to different exogenous (e.g., environmental) and endogenous stimuli  
15 throughout the life cycle of the organism.

Because transcription factors are key controlling elements of biological  
pathways, altering the expression levels of one or more transcription factors can change entire  
biological pathways in an organism. For example, manipulation of the levels of selected  
transcription factors may result in increased expression of economically useful proteins or  
20 metabolic chemicals in plants or to improve other agriculturally relevant characteristics.  
Conversely, blocked or reduced expression of a transcription factor may reduce biosynthesis of  
unwanted compounds or remove an undesirable trait. Therefore, manipulating transcription  
factor levels in a plant offers tremendous potential in agricultural biotechnology for modifying a  
plant's traits.

25

The present invention provides novel transcription factors useful for modifying a  
plant's phenotype in desirable ways, such as modifying a plant's structure or development.

**SUMMARY OF THE INVENTION**

In a first aspect, the invention relates to a recombinant polynucleotide comprising  
a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a  
30 polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-23, or a  
complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding a polypeptide  
comprising a conservatively substituted variant of a polypeptide of (a); (c) a nucleotide sequence  
comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-23, or a

complementary nucleotide sequence thereof; (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c); (e) a nucleotide sequence which hybridizes under stringent conditions over substantially the entire length of a nucleotide sequence of one or more of: (a), (b), (c), or (d); (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e); (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide having a biological activity that modifies a plant's structure and development characteristics; (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g); (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g); (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23; (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23; and (l) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-23. The recombinant polynucleotide may further comprise a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence. The invention also relates to compositions comprising at least two of the above described polynucleotides.

In a second aspect, the invention is an isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide described above.

In another aspect, the invention is a transgenic plant comprising one or more of the above described recombinant polynucleotides. In yet another aspect, the invention is a plant with altered expression levels of a polynucleotide described above or a plant with altered expression or activity levels of an above described polypeptide. Further, the invention is a plant lacking a nucleotide sequence encoding a polypeptide described above. The plant may be a soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, or vegetable brassicas plant.

In a further aspect, the invention relates to a cloning or expression vector comprising the isolated or recombinant polynucleotide described above or cells comprising the cloning or expression vector.

In yet a further aspect, the invention relates to a composition produced by incubating a polynucleotide of the invention with a nuclease, a restriction enzyme, a polymerase; a polymerase and a primer; a cloning vector, or with a cell.

Furthermore, the invention relates to a method for producing a plant having  
5 modified structure and development traits. The method comprises altering the expression of an isolated or recombinant polynucleotide of the invention or altering the expression or activity of a polypeptide of the invention in a plant to produce a modified plant, and selecting the modified plant for modified structure and development traits.

In another aspect, the invention relates to a method of identifying a factor that is  
10 modulated by or interacts with a polypeptide encoded by a polynucleotide of the invention. The method comprises expressing a polypeptide encoded by the polynucleotide in a plant; and identifying at least one factor that is modulated by or interacts with the polypeptide. In one embodiment the method for identifying modulating or interacting factors is by detecting binding by the polypeptide to a promoter sequence, or by detecting interactions between an additional  
15 protein and the polypeptide in a yeast two hybrid system, or by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

In yet another aspect, the invention is a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest. The method comprises placing the molecule in contact with a plant comprising the polynucleotide or  
20 polypeptide encoded by the polynucleotide of the invention and monitoring one or more of the expression level of the polynucleotide in the plant, the expression level of the polypeptide in the plant, and modulation of an activity of the polypeptide in the plant.

In yet another aspect, the invention relates to an integrated system, computer or computer readable medium comprising one or more character strings corresponding to a  
25 polynucleotide of the invention, or to a polypeptide encoded by the polynucleotide. The integrated system, computer or computer readable medium may comprise a link between one or more sequence strings to a modified plant structure and development trait.

In yet another aspect, the invention is a method for identifying a sequence similar or homologous to one or more polynucleotides of the invention, or one or more polypeptides  
30 encoded by the polynucleotides. The method comprises providing a sequence database; and, querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

The method may further comprise of linking the one or more of the polynucleotides of the invention, or encoded polypeptides, to a modified plant structure and development characteristics phenotype.

#### BRIEF DESCRIPTION OF THE DRAWINGS

5 Figure 1 provides a table of exemplary polynucleotide and polypeptide sequences of the invention. The table includes from left to right for each sequence: the SEQ ID No., the internal code reference number (GID), whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

10 Figure 2 provides a table of exemplary sequences that are homologous to other sequences provided in the Sequence Listing and that are derived from *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), identification of the homologous sequence, whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

15 Figure 3 provides a table of exemplary sequences that are homologous to the sequences provided in Figures 1 and 2 and that are derived from plants other than *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), the unique GenBank sequence ID No. (NID), the probability that the comparison was generated by chance (P-value), and the species from which the homologous gene was identified.

20

#### DETAILED DESCRIPTION

The present invention relates to polynucleotides and polypeptides, e.g. for modifying phenotypes of plants.

25 In particular, the polynucleotides or polypeptides are useful for modifying traits associated with a plant's structure or development characteristics when the expression levels of the polynucleotides or expression levels or activity levels of the polypeptides are altered. Specifically, the polynucleotides and polypeptides are useful for modifying the structure and size of flowers, leaves, roots, the plant as a whole, or the like, apical dominance, branching patterns, number of organs, organ identity, whether a plant is sterile or not, the vascularization of a plant, 30 or the developmental staging of a plant, such as when senescence is triggered.

The polynucleotides of the invention encode plant transcription factors. The plant transcription factors are derived, e.g., from *Arabidopsis thaliana* and can belong, e.g., to one or more of the following transcription factor families: the AP2 (APETALA2) domain transcription

factor family (Riechmann and Meyerowitz (1998) J. Biol. Chem. 379:633-646); the MYB transcription factor family (Martin and Paz-Ares (1997) Trends Genet. 13:67-73); the MADS domain transcription factor family (Riechmann and Meyerowitz (1997) J. Biol. Chem. 378:1079-1101); the WRKY protein family (Ishiguro and Nakamura (1994) Mol. Gen. Genet. 244:563-571); the ankyrin-repeat protein family (Zhang et al. (1992) Plant Cell 4:1575-1588); the miscellaneous protein (MISC) family (Kim et al. (1997) Plant J. 11:1237-1251); the zinc finger protein (Z) family (Klug and Schwabe (1995) FASEB J. 9: 597-604); the homeobox (HB) protein family (Duboule (1994) Guidebook to the Homeobox Genes, Oxford University Press); the CAAT-element binding proteins (Forsburg and Guarente (1989) Genes Dev. 3:1166-1178); the squamosa promoter binding proteins (SPB) (Klein et al. (1996) Mol. Gen. Genet. 1996 250:7-16); the NAM protein family; the IAA/AUX proteins (Rouse et al. (1998) Science 279:1371-1373); the HLH/MYC protein family (Littlewood et al. (1994) Prot. Profile 1:639-709); the DNA-binding protein (DBP) family (Tucker et al. (1994) EMBO J. 13:2994-3002); the bZIP family of transcription factors (Foster et al. (1994) FASEB J. 8:192-200); the BPF-1 protein (Box P-binding factor) family (da Costa e Silva et al. (1993) Plant J. 4:125-135); and the golden protein (GLD) family (Hall et al. (1998) Plant Cell 10:925-936).

In addition to methods for modifying a plant phenotype by employing one or more polynucleotides and polypeptides of the invention described herein, the polynucleotides and polypeptides of the invention have a variety of additional uses. These uses include their use in the recombinant production (i.e., expression) of proteins; as regulators of plant gene expression, as diagnostic probes for the presence of complementary or partially complementary nucleic acids (including for detection of natural coding nucleic acids); as substrates for further reactions, e.g., mutation reactions, PCR reactions, or the like, of as substrates for cloning e.g., including digestion or ligation reactions, and for identifying exogenous or endogenous modulators of the transcription factors.

#### DEFINITIONS

A "polynucleotide" is a nucleic acid sequence comprising a plurality of polymerized nucleotide residues, e.g., at least about 15 consecutive polymerized nucleotide residues, optionally at least about 30 consecutive nucleotides, at least about 50 consecutive nucleotides. In many instances, a polynucleotide comprises a nucleotide sequence encoding a polypeptide (or protein) or a domain or fragment thereof. Additionally, the polynucleotide may comprise a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation site, 5' or 3' untranslated regions, a reporter gene, a selectable marker, or the like. The

polynucleotide can be single stranded or double stranded DNA or RNA. The polynucleotide optionally comprises modified bases or a modified backbone. The polynucleotide can be, e.g., genomic DNA or RNA, a transcript (such as an mRNA), a cDNA, a PCR product, a cloned DNA, a synthetic DNA or RNA, or the like. The polynucleotide can comprise a sequence in either  
5 sense or antisense orientations.

A "recombinant polynucleotide" is a polynucleotide that is not in its native state, e.g., the polynucleotide comprises a nucleotide sequence not found in nature, or the polynucleotide is in a context other than that in which it is naturally found, e.g., separated from nucleotide sequences with which it typically is in proximity in nature, or adjacent (or contiguous  
10 with) nucleotide sequences with which it typically is not in proximity. For example, the sequence at issue can be cloned into a vector, or otherwise recombined with one or more additional nucleic acid.

An "isolated polynucleotide" is a polynucleotide whether naturally occurring or recombinant, that is present outside the cell in which it is typically found in nature, whether  
15 purified or not. Optionally, an isolated polynucleotide is subject to one or more enrichment or purification procedures, e.g., cell lysis, extraction, centrifugation, precipitation, or the like.

A "recombinant polypeptide" is a polypeptide produced by translation of a recombinant polynucleotide. An "isolated polypeptide," whether a naturally occurring or a recombinant polypeptide, is more enriched in (or out of) a cell than the polypeptide in its natural  
20 state in a wild type cell, e.g., more than about 5% enriched, more than about 10% enriched, or more than about 20%, or more than about 50%, or more, enriched, i.e., alternatively denoted: 105%, 110%, 120%, 150% or more, enriched relative to wild type standardized at 100%. Such an enrichment is not the result of a natural response of a wild type plant. Alternatively, or additionally, the isolated polypeptide is separated from other cellular components with which it is  
25 typically associated, e.g., by any of the various protein purification methods herein.

The term "transgenic plant" refers to a plant that contains genetic material, not found in a wild type plant of the same species, variety or cultivar. The genetic material may include a transgene, an insertional mutagenesis event (such as by transposon or T-DNA insertional mutagenesis), an activation tagging sequence, a mutated sequence, a homologous  
30 recombination event or a sequence modified by chimeraplasty. Typically, the foreign genetic material has been introduced into the plant by human manipulation.

A transgenic plant may contain an expression vector or cassette. The expression cassette typically comprises a polypeptide-encoding sequence operably linked (i.e., under regulatory control of) to appropriate inducible or constitutive regulatory sequences that allow for

the expression of polypeptide. The expression cassette can be introduced into a plant by transformation or by breeding after transformation of a parent plant. A plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells or any other plant material, e.g., a plant explant, as well as to progeny thereof, and to *in vitro* systems that mimic biochemical or cellular components or processes in a cell.

The phrase "ectopically expression or altered expression" in reference to a polynucleotide indicates that the pattern of expression in, e.g., a transgenic plant or plant tissue, is different from the expression pattern in a wild type plant or a reference plant of the same species. For example, the polynucleotide or polypeptide is expressed in a cell or tissue type other than a cell or tissue type in which the sequence is expressed in the wild type plant, or by expression at a time other than at the time the sequence is expressed in the wild type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at different expression levels (either higher or lower) compared with those found in a wild type plant. The term also refers to altered expression patterns that are produced by lowering the levels of expression to below the detection level or completely abolishing expression. The resulting expression pattern can be transient or stable, constitutive or inducible. In reference to a polypeptide, the term "ectopic expression or altered expression" further may relate to altered activity levels resulting from the interactions of the polypeptides with exogenous or endogenous modulators or from interactions with factors or as a result of the chemical modification of the polypeptides.

The term "fragment" or "domain," with respect to a polypeptide, refers to a subsequence of the polypeptide. In some cases, the fragment or domain, is a subsequence of the polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner, or to a similar extent, as does the intact polypeptide. For example, a polypeptide fragment can comprise a recognizable structural motif or functional domain such as a DNA binding domain that binds to a DNA promoter region, an activation domain or a domain for protein-protein interactions. Fragments can vary in size from as few as 6 amino acids to the full length of the intact polypeptide, but are preferably at least about 30 amino acids in length and more preferably at least about 60 amino acids in length. In reference to a nucleotide sequence, "a fragment" refers to any subsequence of a polynucleotide, typically, of at least consecutive about 15 nucleotides, preferably at least about 30 nucleotides, more preferably at least about 50, of any of the sequences provided herein.

The term "trait" refers to a physiological, morphological, biochemical or physical characteristic of a plant or particular plant material or cell. In some instances, this characteristic is visible to the human eye, such as seed or plant size, or can be measured by available



biochemical techniques, such as the protein, starch or oil content of seed or leaves or by the observation of the expression level of genes, e.g., by employing Northern analysis, RT-PCR, microarray gene expression assays or reporter gene expression systems, or by agricultural observations such as stress tolerance, yield or pathogen tolerance.

5                   “Trait modification” refers to a detectable difference in a characteristic in a plant ectopically expressing a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild type plant. In some cases, the trait modification can be evaluated quantitatively. For example, the trait modification can entail at least about a 2% increase or decrease in an observed trait (difference), at least a 5% difference, at least about a 10%  
10 difference, at least about a 20% difference, at least about a 30%, at least about a 50%, at least about a 70%, or at least about a 100%, or an even greater difference. It is known that there can be a natural variation in the modified trait. Therefore, the trait modification observed entails a change of the normal distribution of the trait in the plants compared with the distribution observed in wild type plant.

15                   Trait modifications of particular interest include those to seed (such as embryo or endosperm), fruit, root, flower, leaf, stem, shoot, seedling or the like, including: enhanced tolerance to environmental conditions including freezing, chilling, heat, drought, water saturation, radiation and ozone; improved tolerance to microbial, fungal or viral diseases; improved tolerance to pest infestations, including nematodes, mollicutes, parasitic higher plants or the like;  
20 decreased herbicide sensitivity; improved tolerance of heavy metals or enhanced ability to take up heavy metals; improved growth under poor photoconditions (e.g., low light and/or short day length), or changes in expression levels of genes of interest. Other phenotype that can be modified relate to the production of plant metabolites, such as variations in the production of taxol, tocopherol, tocotrienol, sterols, phytosterols, vitamins, wax monomers, anti-oxidants,  
25 amino acids, lignins, cellulose, tannins, prenolipids (such as chlorophylls and carotenoids), glucosinolates, and terpenoids, enhanced or compositionally altered protein or oil production (especially in seeds), or modified sugar (insoluble or soluble) and/or starch composition. Physical plant characteristics that can be modified include cell development (such as the number of trichomes), fruit and seed size and number, yields of plant parts such as stems, leaves and  
30 roots, the stability of the seeds during storage, characteristics of the seed pod (e.g., susceptibility to shattering), root hair length and quantity, internode distances, or the quality of seed coat. Plant growth characteristics that can be modified include growth rate, germination rate of seeds, vigor of plants and seedlings, leaf and flower senescence, male sterility, apomixis, flowering time, flower abscission, rate of nitrogen uptake, biomass or transpiration characteristics, as well as

plant architecture characteristics such as apical dominance, branching patterns, number of organs, organ identity, organ shape or size.

#### POLYPEPTIDES AND POLYNUCLEOTIDES OF THE INVENTION

5 The present invention provides, among other things, transcription factors (TFs), and transcription factor homologue polypeptides, and isolated or recombinant polynucleotides encoding the polypeptides. These polypeptides and polynucleotides may be employed to modify a plant's structure and development characteristics.

10 Exemplary polynucleotides encoding the polypeptides of the invention were identified in the *Arabidopsis thaliana* GenBank database using publicly available sequence analysis programs and parameters. Sequences initially identified were then further characterized to identify sequences comprising specified sequence strings corresponding to sequence motifs present in families of known transcription factors. Polynucleotide sequences meeting such criteria were confirmed as transcription factors.

15 Additional polynucleotides of the invention were identified by screening *Arabidopsis thaliana* and/or other plant cDNA libraries with probes corresponding to known transcription factors under low stringency hybridization conditions. Additional sequences, including full length coding sequences were subsequently recovered by the rapid amplification of cDNA ends (RACE) procedure, using a commercially available kit according to the manufacturer's instructions. Where necessary, multiple rounds of RACE are performed to isolate 20 5' and 3' ends. The full length cDNA was then recovered by a routine end-to-end polymerase chain reaction (PCR) using primers specific to the isolated 5' and 3' ends. Exemplary sequences are provided in the Sequence Listing.

The polynucleotides of the invention were ectopically expressed in overexpressor or knockout plants and changes in the structure and development characteristics of the plants 25 were observed. Therefore, the polynucleotides and polypeptides can be employed to improve the structure and development characteristics of plants.

#### Making polynucleotides

30 The polynucleotides of the invention include sequences that encode transcription factors and transcription factor homologue polypeptides and sequences complementary thereto, as well as unique fragments of coding sequence, or sequence complementary thereto. Such polynucleotides can be, e.g., DNA or RNA, e.g., mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, oligonucleotides, etc. The polynucleotides are either double-stranded or single-stranded, and include either, or both sense (i.e., coding) sequences and antisense (i.e., non-

coding, complementary) sequences. The polynucleotides include the coding sequence of a transcription factor, or transcription factor homologue polypeptide, in isolation, in combination with additional coding sequences (e.g., a purification tag, a localization signal, as a fusion-protein, as a pre-protein, or the like), in combination with non-coding sequences (e.g., introns or  
5 inteins, regulatory elements such as promoters, enhancers, terminators, and the like), and/or in a vector or host environment in which the polynucleotide encoding a transcription factor or transcription factor homologue polypeptide is an endogenous or exogenous gene.

A variety of methods exist for producing the polynucleotides of the invention. Procedures for identifying and isolating DNA clones are well known to those of skill in the art, and are described in, e.g., Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, CA ("Berger"); Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 ("Sambrook") and Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing  
10 Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2000) ("Ausubel").

Alternatively, polynucleotides of the invention, can be produced by a variety of in vitro amplification methods adapted to the present invention by appropriate selection of specific or degenerate primers. Examples of protocols sufficient to direct persons of skill through in vitro amplification methods, including the polymerase chain reaction (PCR) the ligase chain  
20 reaction (LCR), Qbeta-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), e.g., for the production of the homologous nucleic acids of the invention are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., (1987) PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) (Innis). Improved methods for cloning in vitro amplified nucleic acids are described in Wallace et al.,  
25 U.S. Pat. No. 5,426,039. Improved methods for amplifying large nucleic acids by PCR are summarized in Cheng et al. (1994) Nature 369: 684-685 and the references cited therein, in which PCR amplicons of up to 40kb are generated. One of skill will appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR expansion and sequencing using reverse transcriptase and a polymerase. See, e.g., Ausubel,  
30 Sambrook and Berger, *all supra*.

Alternatively, polynucleotides and oligonucleotides of the invention can be assembled from fragments produced by solid-phase synthesis methods. Typically, fragments of up to approximately 100 bases are individually synthesized and then enzymatically or chemically ligated to produce a desired sequence, e.g., a polynucleotide encoding all or part of a

transcription factor. For example, chemical synthesis using the phosphoramidite method is described, e.g., by Beaucage et al. (1981) Tetrahedron Letters 22:1859-69; and Matthes et al. (1984) EMBO J. 3:801-5. According to such methods, oligonucleotides are synthesized, purified, annealed to their complementary strand, ligated and then optionally cloned into suitable vectors.

- 5 And if so desired, the polynucleotides and polypeptides of the invention can be custom ordered from any of a number of commercial suppliers.

### HOMOLOGOUS SEQUENCES

- Sequences homologous, i.e., that share significant sequence identity or similarity, to those provided in the Sequence Listing, derived from *Arabidopsis thaliana* or from other plants of choice are also an aspect of the invention. Homologous sequences can be derived from any
- 10 plant including monocots and dicots and in particular agriculturally important plant species, including but not limited to, crops such as soybean, wheat, corn, potato, cotton, rice, oilseed rape (including canola), sunflower, alfalfa, sugarcane and turf; or fruits and vegetables, such as banana, blackberry, blueberry, strawberry, and raspberry, cantaloupe, carrot, cauliflower, coffee,
- 15 cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits (such as apple, peach, pear, cherry and plum) and vegetable brassicas (such as broccoli, cabbage, cauliflower, brussel sprouts and kohlrabi). Other crops, fruits and vegetables whose phenotype can be changed include barley, rye, millet, sorghum, currant, avocado, citrus fruits such as
- 20 oranges, lemons, grapefruit and tangerines, artichoke, cherries, nuts such as the walnut and peanut, endive, leek, roots, such as arrowroot, beet, cassava, turnip, radish, yam, and sweet potato, and beans. The homologous sequences may also be derived from woody species, such as pine, poplar and eucalyptus.

- Transcription factors that are homologous to the listed sequences will typically
- 25 share at least about 30% amino acid sequence identity. More closely related transcription factors can share at least about 50%, about 60%, about 65%, about 70%, about 75% or about 80% or about 90% or about 95% or about 98% or more sequence identity with the listed sequences. Factors that are most closely related to the listed sequences share, e.g., at least about 85%, about 90% or about 95% or more % sequence identity to the listed sequences. At the nucleotide level,
- 30 the sequences will typically share at least about 40% nucleotide sequence identity, preferably at least about 50%, about 60%, about 70% or about 80% sequence identity, and more preferably about 85%, about 90%, about 95% or about 97% or more sequence identity to one or more of the listed sequences. The degeneracy of the genetic code enables major variations in the nucleotide

sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein. Conserved domains within a transcription factor family may exhibit a higher degree of sequence homology, such as at least 65% sequence identity including conservative substitutions, and preferably at least 80% sequence identity.

5

Identifying Nucleic Acids by Hybridization

Polynucleotides homologous to the sequences illustrated in the Sequence Listing can be identified, e.g., by hybridization to each other under stringent or under highly stringent conditions. Single stranded polynucleotides hybridize when they associate based on a variety of well characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. The stringency of a hybridization reflects the degree of sequence identity of the nucleic acids involved, such that the higher the stringency, the more similar are the two polynucleotide strands. Stringency is influenced by a variety of factors, including temperature, salt concentration and composition, organic and non-organic additives, solvents, etc. present in both the hybridization and wash solutions and incubations (and number), as described in more detail in the references cited above.

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An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions, e.g., to a unique subsequence, of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65°C, for example 0.2 x SSC, 0.1% SDS at 65°C. For identification of less closely related homologues washes can be performed at a lower temperature, e.g., 50°C. In general, stringency is increased by raising the wash temperature and/or decreasing the concentration of SSC.

25

As another example, stringent conditions can be selected such that an oligonucleotide that is perfectly complementary to the coding oligonucleotide hybridizes to the coding oligonucleotide with at least about a 5-10x higher signal to noise ratio than the ratio for hybridization of the perfectly complementary oligonucleotide to a nucleic acid encoding a transcription factor known as of the filing date of the application. Conditions can be selected such that a higher signal to noise ratio is observed in the particular assay which is used, e.g., about 15x, 25x, 35x, 50x or more. Accordingly, the subject nucleic acid hybridizes to the unique coding oligonucleotide with at least a 2x higher signal to noise ratio as compared to hybridization

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of the coding oligonucleotide to a nucleic acid encoding known polypeptide. Again, higher signal to noise ratios can be selected, e.g., about 5x, 10x, 25x, 35x, 50x or more. The particular signal will depend on the label used in the relevant assay, e.g., a fluorescent label, a colorimetric label, a radio active label, or the like.

- 5                   Alternatively, transcription factor homologue polypeptides can be obtained by screening an expression library using antibodies specific for one or more transcription factors. With the provision herein of the disclosed transcription factor, and transcription factor homologue nucleic acid sequences, the encoded polypeptide(s) can be expressed and purified in a heterologous expression system (e.g., *E. coli*) and used to raise antibodies (monoclonal or polyclonal) specific for the polypeptide(s) in question. Antibodies can also be raised against synthetic peptides derived from transcription factor, or transcription factor homologue, amino acid sequences. Methods of raising antibodies are well known in the art and are described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Such antibodies can then be used to screen an expression library produced from the plant from which it is desired to clone additional transcription factor homologues, using the methods described above. The selected cDNAs can be confirmed by sequencing and enzymatic activity.
- 10
- 15

#### SEQUENCE VARIATIONS

- It will readily be appreciated by those of skill in the art, that any of a variety of polynucleotide sequences are capable of encoding the transcription factors and transcription factor homologue polypeptides of the invention. Due to the degeneracy of the genetic code, many different polynucleotides can encode identical and/or substantially similar polypeptides in addition to those sequences illustrated in the Sequence Listing.
- 20

- For example, Table 1 illustrates, e.g., that the codons AGC, AGT, TCA, TCC, TCG, and TCT all encode the same amino acid: serine. Accordingly, at each position in the sequence where there is a codon encoding serine, any of the above trinucleotide sequences can be used without altering the encoded polypeptide.
- 25

**Table 1**

Amino acids			Codon							
Alanine	Ala	A	GCA	GCC	GCG	GCU				
Cysteine	Cys	C	TGC	TGT						
Aspartic acid	Asp	D	GAC	GAT						
Glutamic acid	Glu	E	GAA	GAG						
Phenylalanine	Phe	F	TTC	TTT						
Glycine	Gly	G	GGA	GGC	GGG	GGT				
Histidine	His	H	CAC	CAT						
Isoleucine	Ile	I	ATA	ATC	ATT					
Lysine	Lys	K	AAA	AAG						
Leucine	Leu	L	TTA	TTG	CTA	CTC	CTG	CTT		
Methionine	Met	M	ATG							
Asparagine	Asn	N	AAC	AAT						
Proline	Pro	P	CCA	CCC	CCG	CCT				
Glutamine	Gln	Q	CAA	CAG						
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGT		
Serine	Ser	S	AGC	AGT	TCA	TCC	TCG	TCT		
Threonine	Thr	T	ACA	ACC	ACG	ACT				
Valine	Val	V	GTA	GTC	GTG	GTT				
Tryptophan	Trp	W	TGG							
Tyrosine	Tyr	Y	TAC	TAT						

Sequence alterations that do not change the amino acid sequence encoded by the polynucleotide are termed "silent" variations. With the exception of the codons ATG and TGG, encoding methionine and tryptophan, respectively, any of the possible codons for the same amino acid can be substituted by a variety of techniques, e.g., site-directed mutagenesis, available in the art. Accordingly, any and all such variations of a sequence selected from the above table are a feature of the invention.

In addition to silent variations, other conservative variations that alter one, or a few amino acids in the encoded polypeptide, can be made without altering the function of the polypeptide, these conservative variants are, likewise, a feature of the invention.

For example, substitutions, deletions and insertions introduced into the sequences provided in the Sequence Listing are also envisioned by the invention. Such sequence modifications can be engineered into a sequence by site-directed mutagenesis (Wu (ed.) Meth. Enzymol. (1993) vol. 217, Academic Press) or the other methods noted below. Amino acid substitutions are typically of single residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. In preferred embodiments, deletions or insertions are made in adjacent pairs, e.g., a deletion of two residues or insertion of two residues. Substitutions, deletions, insertions or any combination thereof can be

combined to arrive at a sequence. The mutations that are made in the polynucleotide encoding the transcription factor should not place the sequence out of reading frame and should not create complementary regions that could produce secondary mRNA structure. Preferably, the polypeptide encoded by the DNA performs the desired function.

- 5                   Conservative substitutions are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the Table 2 when it is desired to maintain the activity of the protein. Table 2 shows amino acids which can be substituted for an amino acid in a protein and which are typically regarded as conservative substitutions.

10

**Table 2**

<b>Residue</b>	<b>Conservative Substitutions</b>
Ala	Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Gln	Asn
Cys	Ser
Glu	Asp
Gly	Pro
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr; Gly
Thr	Ser; Val
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Substitutions that are less conservative than those in Table 2 can be selected by picking residues that differ more significantly in their effect on maintaining (a) the structure of



the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

10 FURTHER MODIFYING SEQUENCES OF THE INVENTION—MUTATION/ FORCED EVOLUTION

In addition to generating silent or conservative substitutions as noted, above, the present invention optionally includes methods of modifying the sequences of the Sequence Listing. In the methods, nucleic acid or protein modification methods are used to alter the given sequences to produce new sequences and/or to chemically or enzymatically modify given sequences to change the properties of the nucleic acids or proteins.

Thus, in one embodiment, given nucleic acid sequences are modified, e.g., according to standard mutagenesis or artificial evolution methods to produce modified sequences. For example, Ausubel, *supra*, provides additional details on mutagenesis methods. Artificial forced evolution methods are described, e.g., by Stemmer (1994) *Nature* 370:389-391, and Stemmer (1994) *Proc. Natl. Acad. Sci. USA* 91:10747-10751. Many other mutation and evolution methods are also available and expected to be within the skill of the practitioner.

Similarly, chemical or enzymatic alteration of expressed nucleic acids and polypeptides can be performed by standard methods. For example, sequence can be modified by addition of lipids, sugars, peptides, organic or inorganic compounds, by the inclusion of modified nucleotides or amino acids, or the like. For example, protein modification techniques are illustrated in Ausubel, *supra*. Further details on chemical and enzymatic modifications can be found herein. These modification methods can be used to modify any given sequence, or to modify any sequence produced by the various mutation and artificial evolution modification methods noted herein.

Accordingly, the invention provides for modification of any given nucleic acid by mutation, evolution, chemical or enzymatic modification, or other available methods, as well as for the products produced by practicing such methods, e.g., using the sequences herein as a starting substrate for the various modification approaches.

For example, optimized coding sequence containing codons preferred by a particular prokaryotic or eukaryotic host can be used e.g., to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced using a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, preferred stop codons for *S. cerevisiae* and mammals are TAA and TGA, respectively. The preferred stop codon for monocotyledonous plants is TGA, whereas insects and *E. coli* prefer to use TAA as the stop codon.

The polynucleotide sequences of the present invention can also be engineered in order to alter a coding sequence for a variety of reasons, including but not limited to, alterations which modify the sequence to facilitate cloning, processing and/or expression of the gene product. For example, alterations are optionally introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

Furthermore, a fragment or domain derived from any of the polypeptides of the invention can be combined with domains derived from other transcription factors or synthetic domains to modify the biological activity of a transcription factor. For instance, a DNA binding domain derived from a transcription factor of the invention can be combined with the activation domain of another transcription factor or with a synthetic activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) Proc. Natl. Acad. Sci. USA 95: 376-381; and Aoyama et al. (1995) Plant Cell 7:1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51: 113-119) and synthetic peptides (Giniger and Ptashne, (1987) Nature 330:670-672).

## 25 EXPRESSION AND MODIFICATION OF POLYPEPTIDES

Typically, polynucleotide sequences of the invention are incorporated into recombinant DNA (or RNA) molecules that direct expression of polypeptides of the invention in appropriate host cells, transgenic plants, in vitro translation systems, or the like. Due to the inherent degeneracy of the genetic code, nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence can be substituted for any listed sequence to provide for cloning and expressing the relevant homologue.

### Vectors, Promoters and Expression Systems

The present invention includes recombinant constructs comprising one or more of the nucleic acid sequences herein. The constructs typically comprise a vector, such as a plasmid, a cosmid, a phage, a virus (e.g., a plant virus), a bacterial artificial chromosome (BAC),  
5 a yeast artificial chromosome (YAC), or the like, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

10 General texts which describe molecular biological techniques useful herein, including the use and production of vectors, promoters and many other relevant topics, include Berger, Sambrook and Ausubel, *supra*. Any of the identified sequences can be incorporated into a cassette or vector, e.g., for expression in plants. A number of expression vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described  
15 including those described in Weissbach and Weissbach, (1989) Methods for Plant Molecular Biology, Academic Press, and Gelvin et al., (1990) Plant Molecular Biology Manual, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed by Herrera-Estrella et al. (1983) Nature 303: 209, Bevan (1984) Nucl Acid Res. 12: 8711-8721, Klee (1985) Bio/Technology 3: 637-642,  
20 for dicotyledonous plants.

Alternatively, non-Ti vectors can be used to transfer the DNA into monocotyledonous plants and cells by using free DNA delivery techniques. Such methods can involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon  
carbide whiskers, and viruses. By using these methods transgenic plants such as wheat, rice  
25 (Christou (1991) Bio/Technology 9: 957-962) and corn (Gordon-Kamm (1990) Plant Cell 2: 603-618) can be produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks et al. (1993) Plant Physiol 102: 1077-1084; Vasil (1993) Bio/Technology 10: 667-674; Wan and Lemeaux (1994) Plant Physiol 104: 37-48, and for *Agrobacterium*-mediated DNA transfer (Ishida et al. (1996) Nature Biotech  
30 14: 745-750).

Typically, plant transformation vectors include one or more cloned plant coding sequence (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter (e.g., a regulatory region controlling inducible or constitutive, environmentally-or

developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

- Examples of constitutive plant promoters which can be useful for expressing the
- 5 TF sequence include: the cauliflower mosaic virus (CaMV) 35S promoter, which confers constitutive, high-level expression in most plant tissues (*see, e.g., Odel et al. (1985) Nature 313:810*); the nopaline synthase promoter (An et al. (1988) Plant Physiol 88:547); and the octopine synthase promoter (Fromm et al. (1989) Plant Cell 1: 977).

- A variety of plant gene promoters that regulate gene expression in response to
- 10 environmental, hormonal, chemical, developmental signals, and in a tissue-active manner can be used for expression of a TF sequence in plants. Choice of a promoter is based largely on the phenotype of interest and is determined by such factors as tissue (e.g., seed, fruit, root, pollen, vascular tissue, flower, carpel, etc.), inducibility (e.g., in response to wounding, heat, cold, drought, light, pathogens, etc.), timing, developmental stage, and the like. Numerous known
- 15 promoters have been characterized and can favorably be employed to promote expression of a polynucleotide of the invention in a transgenic plant or cell of interest. For example, tissue specific promoters include: seed-specific promoters (such as the napin, phaseolin or DC3 promoter described in US Pat. No. 5,773,697), fruit-specific promoters that are active during fruit ripening (such as the dru 1 promoter (US Pat. No. 5,783,393), or the 2A11 promoter (US Pat. No.
- 20 4,943,674) and the tomato polygalacturonase promoter (Bird et al. (1988) Plant Mol Biol 11:651), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186, pollen-active promoters such as PTA29, PTA26 and PTA13 (US Pat. No. 5,792,929), promoters active in vascular tissue (Ringli and Keller (1998) Plant Mol Biol 37:977-988), flower-specific (Kaiser et al, (1995) Plant Mol Biol 28:231-243), pollen (Baerson et al. (1994) Plant Mol
- 25 Biol 26:1947-1959), carpels (Ohl et al. (1990) Plant Cell 2:837-848), pollen and ovules (Baerson et al. (1993) Plant Mol Biol 22:255-267), auxin-inducible promoters (such as that described in van der Kop et al. (1999) Plant Mol Biol 39:979-990 or Baumann et al. (1999) Plant Cell 11:323-334), cytokinin-inducible promoter (Guevara-Garcia (1998) Plant Mol Biol 38:743-753), promoters responsive to gibberellin (Shi et al. (1998) Plant Mol Biol 38:1053-1060, Willmott et
- 30 al. (1998) 38:817-825) and the like. Additional promoters are those that elicit expression in response to heat (Ainley et al. (1993) Plant Mol Biol 22: 13-23), light (e.g., the pea rbcS-3A promoter, Kuhlmeier et al. (1989) Plant Cell 1:471, and the maize rbcS promoter, Schaffner and Sheen (1991) Plant Cell 3: 997); wounding (e.g., *wun1*, Siebertz et al. (1989) Plant Cell 1: 961); pathogens (such as the PR-1 promoter described in Buchel et al. (1999) Plant Mol. Biol. 40:387-

396, and the PDF1.2 promoter described in Manners et al. (1998) Plant Mol. Biol. 38:1071-80), and chemicals such as methyl jasmonate or salicylic acid (Gatz et al. (1997) Plant Mol Biol 48: 89-108). In addition, the timing of the expression can be controlled by using promoters such as those acting at senescence (An and Amazon (1995) Science 270: 1986-1988); or late seed development  
5 (Odell et al. (1994) Plant Physiol 106:447-458).

Plant expression vectors can also include RNA processing signals that can be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors can include additional regulatory sequences from the 3'-untranslated region of plant genes, e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II  
10 terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

#### Additional Expression Elements

Specific initiation signals can aid in efficient translation of coding sequences. These signals can include, e.g., the ATG initiation codon and adjacent sequences. In cases where a coding sequence, its initiation codon and upstream sequences are inserted into the appropriate  
15 expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence (e.g., a mature protein coding sequence), or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon can be separately provided. The initiation codon is provided in the correct reading frame to facilitate transcription. Exogenous transcriptional elements and initiation codons can be of various origins,  
20 both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use.

#### Expression Hosts

The present invention also relates to host cells which are transduced with vectors of the invention, and the production of polypeptides of the invention (including fragments  
25 thereof) by recombinant techniques. Host cells are genetically engineered (i.e, nucleic acids are introduced, e.g., transduced, transformed or transfected) with the vectors of this invention, which may be, for example, a cloning vector or an expression vector comprising the relevant nucleic acids herein. The vector is optionally a plasmid, a viral particle, a phage, a naked nucleic acids, etc. The engineered host cells can be cultured in conventional nutrient media modified as  
30 appropriate for activating promoters, selecting transformants, or amplifying the relevant gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art and in the references cited herein, including, Sambrook and Ausubel.

The host cell can be a eukaryotic cell, such as a yeast cell, or a plant cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Plant protoplasts are also suitable for some applications. For example, the DNA fragments are introduced into plant tissues, cultured plant cells or plant protoplasts by standard methods including electroporation (Fromm et al., (1985) Proc. Natl. Acad. Sci. USA 82, 5824, infection by viral vectors such as cauliflower mosaic virus (CaMV) (Hohn et al., (1982) Molecular Biology of Plant Tumors, (Academic Press, New York) pp. 549-560; US 4,407,956), high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., (1987) Nature 327, 70-73), use of pollen as vector (WO 85/01856), or use of *Agrobacterium tumefaciens* or *A. rhizogenes* carrying a T-DNA plasmid in which DNA fragments are cloned. The T-DNA plasmid is transmitted to plant cells upon infection by *Agrobacterium tumefaciens*, and a portion is stably integrated into the plant genome (Horsch et al. (1984) Science 233:496-498; Fraley et al. (1983) Proc. Natl. Acad. Sci. USA 80, 4803).

The cell can include a nucleic acid of the invention which encodes a polypeptide, wherein the cells expresses a polypeptide of the invention. The cell can also include vector sequences, or the like. Furthermore, cells and transgenic plants which include any polypeptide or nucleic acid above or throughout this specification, e.g., produced by transduction of a vector of the invention, are an additional feature of the invention.

For long-term, high-yield production of recombinant proteins, stable expression can be used. Host cells transformed with a nucleotide sequence encoding a polypeptide of the invention are optionally cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein or fragment thereof produced by a recombinant cell may be secreted, membrane-bound, or contained intracellularly, depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding mature proteins of the invention can be designed with signal sequences which direct secretion of the mature polypeptides through a prokaryotic or eukaryotic cell membrane.

#### Modified Amino Acids

Polypeptides of the invention may contain one or more modified amino acids.

The presence of modified amino acids may be advantageous in, for example, increasing polypeptide half-life, reducing polypeptide antigenicity or toxicity, increasing polypeptide storage stability, or the like. Amino acid(s) are modified, for example, co-translationally or post-translationally during recombinant production or modified by synthetic or chemical means.

Non-limiting examples of a modified amino acid include incorporation or other use of acetylated amino acids, glycosylated amino acids, sulfated amino acids, prenylated (e.g., farnesylated, geranylgeranylated) amino acids, PEG modified (e.g., "PEGylated") amino acids, biotinylated amino acids, carboxylated amino acids, phosphorylated amino acids, etc. References  
5 adequate to guide one of skill in the modification of amino acids are replete throughout the literature.

#### IDENTIFICATION OF ADDITIONAL FACTORS

A transcription factor provided by the present invention can also be used to identify additional endogenous or exogenous molecules that can affect a phenotype or trait of  
10 interest. On the one hand, such molecules include organic (small or large molecules) and/or inorganic compounds that affect expression of (i.e., regulate) a particular transcription factor. Alternatively, such molecules include endogenous molecules that are acted upon either at a transcriptional level by a transcription factor of the invention to modify a phenotype as desired. For example, the transcription factors can be employed to identify one or more downstream gene  
15 with which is subject to a regulatory effect of the transcription factor. In one approach, a transcription factor or transcription factor homologue of the invention is expressed in a host cell, e.g. a transgenic plant cell, tissue or explant, and expression products, either RNA or protein, of likely or random targets are monitored, e.g., by hybridization to a microarray of nucleic acid probes corresponding to genes expressed in a tissue or cell type of interest, by two-dimensional  
20 gel electrophoresis of protein products, or by any other method known in the art for assessing expression of gene products at the level of RNA or protein. Alternatively, a transcription factor of the invention can be used to identify promoter sequences (i.e., binding sites) involved in the regulation of a downstream target. After identifying a promoter sequence, interactions between the transcription factor and the promoter sequence can be modified by changing specific  
25 nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences can be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the transcription factors with their promoters (Bulyk et al.  
30 (1999) Nature Biotechnology 17:573-577).

The identified transcription factors are also useful to identify proteins that modify the activity of the transcription factor. Such modification can occur by covalent modification, such as by phosphorylation, or by protein-protein (homo or-heteropolymer) interactions. Any

method suitable for detecting protein-protein interactions can be employed. Among the methods that can be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns, and the two-hybrid yeast system.

The two-hybrid system detects protein interactions in vivo and is described in Chien, et al., (1991), Proc. Natl. Acad. Sci. USA 88, 9578-9582 and is commercially available from Clontech (Palo Alto, Calif.). In such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the TF polypeptide and the other consists of the transcription activator protein's activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into the plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product. Then, the library plasmids responsible for reporter gene expression are isolated and sequenced to identify the proteins encoded by the library plasmids. After identifying proteins that interact with the transcription factors, assays for compounds that interfere with the TF protein-protein interactions can be preformed.

## 20 IDENTIFICATION OF MODULATORS

In addition to the intracellular molecules described above, extracellular molecules that alter activity or expression of a transcription factor, either directly or indirectly, can be identified. For example, the methods can entail first placing a candidate molecule in contact with a plant or plant cell. The molecule can be introduced by topical administration, such as spraying or soaking of a plant, and then the molecule's effect on the expression or activity of the TF polypeptide or the expression of the polynucleotide monitored. Changes in the expression of the TF polypeptide can be monitored by use of polyclonal or monoclonal antibodies, gel electrophoresis or the like. Changes in the expression of the corresponding polynucleotide sequence can be detected by use of microarrays, Northern, quantitative PCR, or any other technique for monitoring changes in mRNA expression. These techniques are exemplified in Ausubel et al. (eds) Current Protocols in Molecular Biology, John Wiley & Sons (1998). Such changes in the expression levels can be correlated with modified plant traits and thus identified



molecules can be useful for soaking or spraying on fruit, vegetable and grain crops to modify traits in plants.

Essentially any available composition can be tested for modulatory activity of expression or activity of any nucleic acid or polypeptide herein. Thus, available libraries of compounds such as chemicals, polypeptides, nucleic acids and the like can be tested for modulatory activity. Often, potential modulator compounds can be dissolved in aqueous or organic (e.g., DMSO-based) solutions for easy delivery to the cell or plant of interest in which the activity of the modulator is to be tested. Optionally, the assays are designed to screen large modulator composition libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays).

In one embodiment, high throughput screening methods involve providing a combinatorial library containing a large number of potential compounds (potential modulator compounds). Such "combinatorial chemical libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as target compounds.

A combinatorial chemical library can be, e.g., a collection of diverse chemical compounds generated by chemical synthesis or biological synthesis. For example, a combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (e.g., in one example, amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound of a set length). Exemplary libraries include peptide libraries, nucleic acid libraries, antibody libraries (see, e.g., Vaughn et al. (1996) *Nature Biotechnology*, 14(3):309-314 and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al. *Science* (1996) 274:1520-1522 and U.S. Patent 5,593,853), peptide nucleic acid libraries (see, e.g., U.S. Patent 5,539,083), and small organic molecule libraries (see, e.g., benzodiazepines, Baum *C&EN* Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337) and the like.

Preparation and screening of combinatorial or other libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent 5,010,175, Furka, *Int. J. Pept. Prot. Res.* 37:487-493 (1991) and Houghton et al. *Nature* 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used.

In addition, as noted, compound screening equipment for high-throughput screening is generally available, e.g., using any of a number of well known robotic systems that have also been developed for solution phase chemistries useful in assay systems. These systems include automated workstations including an automated synthesis apparatus and robotic systems  
5 utilizing robotic arms. Any of the above devices are suitable for use with the present invention, e.g., for high-throughput screening of potential modulators. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

Indeed, entire high throughput screening systems are commercially available.  
10 These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. Similarly, microfluidic implementations of screening are also commercially available.

15 The manufacturers of such systems provide detailed protocols the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like. The integrated systems herein, in addition to providing for sequence alignment and, optionally, synthesis of relevant nucleic acids, can include such screening apparatus to identify modulators  
20 that have an effect on one or more polynucleotides or polypeptides according to the present invention.

In some assays it is desirable to have positive controls to ensure that the components of the assays are working properly. At least two types of positive controls are appropriate. That is, known transcriptional activators or inhibitors can be incubated with  
25 cells/plants/ etc. in one sample of the assay, and the resulting increase/decrease in transcription can be detected by measuring the resulting increase in RNA/ protein expression, etc., according to the methods herein. It will be appreciated that modulators can also be combined with transcriptional activators or inhibitors to find modulators which inhibit transcriptional activation or transcriptional repression. Either expression of the nucleic acids and proteins herein or any  
30 additional nucleic acids or proteins activated by the nucleic acids or proteins herein, or both, can be monitored.

In an embodiment, the invention provides a method for identifying compositions that modulate the activity or expression of a polynucleotide or polypeptide of the invention. For example, a test compound, whether a small or large molecule, is placed in contact with a cell,

plant (or plant tissue or explant), or composition comprising the polynucleotide or polypeptide of interest and a resulting effect on the cell, plant, (or tissue or explant) or composition is evaluated by monitoring, either directly or indirectly, one or more of: expression level of the polynucleotide or polypeptide, activity (or modulation of the activity) of the polynucleotide or polypeptide. In some cases, an alteration in a plant phenotype can be detected following contact of a plant (or plant cell, or tissue or explant) with the putative modulator, e.g., by modulation of expression or activity of a polynucleotide or polypeptide of the invention.

#### SUBSEQUENCES

Also contemplated are uses of polynucleotides, also referred to herein as oligonucleotides, typically having at least 12 bases, preferably at least 15, more preferably at least 20, 30, or 50 bases, which hybridize under at least highly stringent (or ultra-high stringent or ultra-ultra- high stringent conditions) conditions to a polynucleotide sequence described above. The polynucleotides may be used as probes, primers, sense and antisense agents, and the like, according to methods as noted *supra*.

Subsequences of the polynucleotides of the invention, including polynucleotide fragments and oligonucleotides are useful as nucleic acid probes and primers. An oligonucleotide suitable for use as a probe or primer is at least about 15 nucleotides in length, more often at least about 18 nucleotides, often at least about 21 nucleotides, frequently at least about 30 nucleotides, or about 40 nucleotides, or more in length. A nucleic acid probe is useful in hybridization protocols, e.g., to identify additional polypeptide homologues of the invention, including protocols for microarray experiments. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods. See Sambrook and Ausubel, *supra*.

In addition, the invention includes an isolated or recombinant polypeptide including a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotides of the invention. For example, such polypeptides, or domains or fragments thereof, can be used as immunogens, e.g., to produce antibodies specific for the polypeptide sequence, or as probes for detecting a sequence of interest. A subsequence can range in size from about 15 amino acids in length up to and including the full length of the polypeptide.

## PRODUCTION OF TRANSGENIC PLANTS

### Modification of Traits

The polynucleotides of the invention are favorably employed to produce transgenic plants with various traits, or characteristics, that have been modified in a desirable manner, e.g., to improve the seed characteristics of a plant. For example, alteration of expression levels or patterns (e.g., spatial or temporal expression patterns) of one or more of the transcription factors (or transcription factor homologues) of the invention, as compared with the levels of the same protein found in a wild type plant, can be used to modify a plant's traits. An illustrative example of trait modification, modified structure and development characteristics, by altering expression levels of a particular transcription factor is described further in the Examples and the Sequence Listing.

### Antisense and Cosuppression Approaches

In addition to expression of the nucleic acids of the invention as gene replacement or plant phenotype modification nucleic acids, the nucleic acids are also useful for sense and anti-sense suppression of expression, e.g., to down-regulate expression of a nucleic acid of the invention, e.g., as a further mechanism for modulating plant phenotype. That is, the nucleic acids of the invention, or subsequences or anti-sense sequences thereof, can be used to block expression of naturally occurring homologous nucleic acids. A variety of sense and anti-sense technologies are known in the art, e.g., as set forth in Lichtenstein and Nellen (1997) Antisense Technology: A Practical Approach IRL Press at Oxford University, Oxford, England. In general, sense or anti-sense sequences are introduced into a cell, where they are optionally amplified, e.g., by transcription. Such sequences include both simple oligonucleotide sequences and catalytic sequences such as ribozymes.

For example, a reduction or elimination of expression (i.e., a "knock-out") of a transcription factor or transcription factor homologue polypeptide in a transgenic plant, e.g., to modify a plant trait, can be obtained by introducing an antisense construct corresponding to the polypeptide of interest as a cDNA. For antisense suppression, the transcription factor or homologue cDNA is arranged in reverse orientation (with respect to the coding sequence) relative to the promoter sequence in the expression vector. The introduced sequence need not be the full length cDNA or gene, and need not be identical to the cDNA or gene found in the plant type to be transformed. Typically, the antisense sequence need only be capable of hybridizing to the target gene or RNA of interest. Thus, where the introduced sequence is of shorter length, a higher degree of homology to the endogenous transcription factor sequence will be needed for effective antisense suppression. While antisense sequences of various lengths can be utilized, preferably,

the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of

5 RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous transcription factor gene in the plant cell.

Suppression of endogenous transcription factor gene expression can also be achieved using a ribozyme. Ribozymes are RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No.

10 4,987,071 and U.S. Patent No. 5,543,508. Synthetic ribozyme sequences including antisense RNAs can be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that hybridize to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

Vectors in which RNA encoded by a transcription factor or transcription factor

15 homologue cDNA is over-expressed can also be used to obtain co-suppression of a corresponding endogenous gene, e.g., in the manner described in U.S. Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire transcription factor cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous transcription factor gene of interest. However, as with

20 antisense suppression, the suppressive efficiency will be enhanced as specificity of hybridization is increased, e.g., as the introduced sequence is lengthened, and/or as the sequence similarity between the introduced sequence and the endogenous transcription factor gene is increased.

Vectors expressing an untranslatable form of the transcription factor mRNA, e.g., sequences comprising one or more stop codon, or nonsense mutation) can also be used to

25 suppress expression of an endogenous transcription factor, thereby reducing or eliminating it's activity and modifying one or more traits. Methods for producing such constructs are described in U.S. Patent No. 5,583,021. Preferably, such constructs are made by introducing a premature stop codon into the transcription factor gene. Alternatively, a plant trait can be modified by gene silencing using double-strand RNA (Sharp (1999) Genes and Development 13: 139-141).

30 Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a transcription factor or transcription factor homologue gene. Plants containing a single transgene insertion

event at the desired gene can be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) Methods in Arabidopsis Research, World Scientific).

Alternatively, a plant phenotype can be altered by eliminating an endogenous gene, such as a transcription factor or transcription factor homologue, e.g., by homologous recombination (Kempin et al. (1997) Nature 389:802).

A plant trait can also be modified by using the cre-lox system (for example, as described in US Pat. No. 5,658,772). A plant genome can be modified to include first and second lox sites that are then contacted with a Cre recombinase. If the lox sites are in the same orientation, the intervening DNA sequence between the two sites is excised. If the lox sites are in the opposite orientation, the intervening sequence is inverted.

The polynucleotides and polypeptides of this invention can also be expressed in a plant in the absence of an expression cassette by manipulating the activity or expression level of the endogenous gene by other means. For example, by ectopically expressing a gene by T-DNA activation tagging (Ichikawa et al. (1997) Nature 390 698-701; Kakimoto et al. (1996) Science 274: 982-985). This method entails transforming a plant with a gene tag containing multiple transcriptional enhancers and once the tag has inserted into the genome, expression of a flanking gene coding sequence becomes deregulated. In another example, the transcriptional machinery in a plant can be modified so as to increase transcription levels of a polynucleotide of the invention (See, e.g., PCT Publications WO 96/06166 and WO 98/53057 which describe the modification of the DNA binding specificity of zinc finger proteins by changing particular amino acids in the DNA binding motif).

The transgenic plant can also include the machinery necessary for expressing or altering the activity of a polypeptide encoded by an endogenous gene, for example by altering the phosphorylation state of the polypeptide to maintain it in an activated state.

Transgenic plants (or plant cells, or plant explants, or plant tissues) incorporating the polynucleotides of the invention and/or expressing the polypeptides of the invention can be produced by a variety of well established techniques as described above. Following construction of a vector, most typically an expression cassette, including a polynucleotide, e.g., encoding a transcription factor or transcription factor homologue, of the invention, standard techniques can be used to introduce the polynucleotide into a plant, a plant cell, a plant explant or a plant tissue of interest. Optionally, the plant cell, explant or tissue can be regenerated to produce a transgenic plant.

The plant can be any higher plant, including gymnosperms, monocotyledonous and dicotyledonous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean,

- clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcubitaceae* (melons and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.), *Solanaceae* (potato, tomato, tobacco, peppers, etc.), and various other crops. See protocols described in Ammirato et al. (1984) Handbook of Plant Cell Culture –Crop Species.
- 5 Macmillan Publ. Co. Shimamoto et al. (1989) Nature 338:274-276; Fromm et al. (1990) Bio/Technology 8:833-839; and Vasil et al. (1990) Bio/Technology 8:429-434.

Transformation and regeneration of both monocotyledonous and dicotyledonous plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be

10 transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods can include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and *Agrobacterium tumefaciens* mediated

15 transformation. Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence.

Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, and which are herein incorporated by reference, include: U.S. Patent Nos.

20 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

Following transformation, plants are preferably selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants

25 can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

After transformed plants are selected and grown to maturity, those plants showing a modified trait are identified. The modified trait can be any of those traits described above. Additionally, to confirm that the modified trait is due to changes in expression levels or

30 activity of the polypeptide or polynucleotide of the invention can be determined by analyzing mRNA expression using Northern blots, RT-PCR or microarrays, or protein expression using immunoblots or Western blots or gel shift assays.

### INTEGRATED SYSTEMS—SEQUENCE IDENTITY

Additionally, the present invention may be an integrated system, computer or computer readable medium that comprises an instruction set for determining the identity of one or more sequences in a database. In addition, the instruction set can be used to generate or identify sequences that meet any specified criteria. Furthermore, the instruction set may be used to associate or link certain functional benefits, such modified structure and development characteristics, with one or more identified sequence.

For example, the instruction set can include, e.g., a sequence comparison or other alignment program, e.g., an available program such as, for example, the Wisconsin Package Version 10.0, such as BLAST, FASTA, PILEUP, FINDPATTERNS or the like (GCG, Madison, WI). Public sequence databases such as GenBank, EMBL, Swiss-Prot and PIR or private sequence databases such as PhytoSeq (Incyte Pharmaceuticals, Palo Alto, CA) can be searched.

Alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85: 2444, by computerized implementations of these algorithms. After alignment, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a comparison window to identify and compare local regions of sequence similarity. The comparison window can be a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 contiguous positions. A description of the method is provided in Ausubel et al., *supra*.

A variety of methods of determining sequence relationships can be used, including manual alignment and computer assisted sequence alignment and analysis. This later approach is a preferred approach in the present invention, due to the increased throughput afforded by computer assisted methods. As noted above, a variety of computer programs for performing sequence alignment are available, or can be produced by one of skill.

One example algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is



referred to as the neighborhood word score threshold (Altschul et al., *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for  
5 nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of  
10 one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E)  
15 of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see*, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided  
20 by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence (and, therefore, in this context, homologous) if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, or less than about 0.01, and or  
25 even less than about 0.001. An additional example of a useful sequence alignment algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. The program can align, e.g., up to 300 sequences of a maximum length of 5,000 letters.

The integrated system, or computer typically includes a user input interface  
30 allowing a user to selectively view one or more sequence records corresponding to the one or more character strings, as well as an instruction set which aligns the one or more character strings with each other or with an additional character string to identify one or more region of sequence similarity. The system may include a link of one or more character strings with a particular

phenotype or gene function. Typically, the system includes a user readable output element which displays an alignment produced by the alignment instruction set.

The methods of this invention can be implemented in a localized or distributed computing environment. In a distributed environment, the methods may implemented on a single  
5 computer comprising multiple processors or on a multiplicity of computers. The computers can be linked, e.g. through a common bus, but more preferably the computer(s) are nodes on a network. The network can be a generalized or a dedicated local or wide-area network and, in certain preferred embodiments, the computers may be components of an intra-net or an internet.

Thus, the invention provides methods for identifying a sequence similar or  
10 homologous to one or more polynucleotides as noted herein, or one or more target polypeptides encoded by the polynucleotides, or otherwise noted herein and may include linking or associating a given plant phenotype or gene function with a sequence. In the methods, a sequence database is provided (locally or across an inter or intra net) and a query is made against the sequence database using the relevant sequences herein and associated plant phenotypes or gene functions.

Any sequence herein can be entered into the database, before or after querying  
15 the database. This provides for both expansion of the database and, if done before the querying step, for insertion of control sequences into the database. The control sequences can be detected by the query to ensure the general integrity of both the database and the query. As noted, the query can be performed using a web browser based interface. For example, the database can be a  
20 centralized public database such as those noted herein, and the querying can be done from a remote terminal or computer across an internet or intranet.

### EXAMPLES

The following examples are intended to illustrate but not limit the present invention.

#### 25 EXAMPLE I. FULL LENGTH GENE IDENTIFICATION AND CLONING

Putative transcription factor sequences (genomic or ESTs) related to known transcription factors were identified in the *Arabidopsis thaliana* GenBank database using the tblastn sequence analysis program using default parameters and a P-value cutoff threshold of -4 or -5 or lower, depending on the length of the query sequence. Putative transcription factor  
30 sequence hits were then screened to identify those containing particular sequence strings. If the sequence hits contained such sequence strings, the sequences were confirmed as transcription factors.

Alternatively, *Arabidopsis thaliana* cDNA libraries derived from different tissues or treatments, or genomic libraries were screened to identify novel members of a transcription family using a low stringency hybridization approach. Probes were synthesized using gene specific primers in a standard PCR reaction (annealing temperature 60°C) and labeled with <sup>32</sup>P dCTP using the High Prime DNA Labeling Kit (Boehringer Mannheim). Purified radiolabelled probes were added to filters immersed in Church hybridization medium (0.5 M NaPO<sub>4</sub> pH 7.0, 7% SDS, 1 % w/v bovine serum albumin) and hybridized overnight at 60 °C with shaking. Filters were washed two times for 45 to 60 minutes with 1xSCC, 1% SDS at 60°C.

To identify additional sequence 5' or 3' of a partial cDNA sequence in a cDNA library, 5' and 3' rapid amplification of cDNA ends (RACE) was performed using the Marathon™ cDNA amplification kit (Clontech, Palo Alto, CA). Generally, the method entailed first isolating poly(A) mRNA, performing first and second strand cDNA synthesis to generate double stranded cDNA, blunting cDNA ends, followed by ligation of the Marathon™ Adaptor to the cDNA to form a library of adaptor-ligated ds cDNA.

Gene-specific primers were designed to be used along with adaptor specific primers for both 5' and 3' RACE reactions. Nested primers, rather than single primers, were used to increase PCR specificity. Using 5' and 3' RACE reactions, 5' and 3' RACE fragments were obtained, sequenced and cloned. The process can be repeated until 5' and 3' ends of the full-length gene were identified. Then the full-length cDNA was generated by PCR using primers specific to 5' and 3' ends of the gene by end-to-end PCR.

#### EXAMPLE II. CONSTRUCTION OF EXPRESSION VECTORS

The sequence was amplified from a genomic or cDNA library using primers specific to sequences upstream and downstream of the coding region. The expression vector was pMEN20 or pMEN65, which are both derived from pMON316 (Sanders et al, (1987) Nucleic Acids Research 15:1543-58) and contain the CaMV 35S promoter to express transgenes. To clone the sequence into the vector, both pMEN20 and the amplified DNA fragment were digested separately with SalI and NotI restriction enzymes at 37° C for 2 hours. The digestion products were subject to electrophoresis in a 0.8% agarose gel and visualized by ethidium bromide staining. The DNA fragments containing the sequence and the linearized plasmid were excised and purified by using a Qiaquick gel extraction kit (Qiagen, CA). The fragments of interest were ligated at a ratio of 3:1 (vector to insert). Ligation reactions using T4 DNA ligase (New England Biolabs, MA) were carried out at 16° C for 16 hours. The ligated DNAs were transformed into

competent cells of the *E. coli* strain DH5alpha by using the heat shock method. The transformations were plated on LB plates containing 50 mg/l kanamycin (Sigma).

- Individual colonies were grown overnight in five milliliters of LB broth containing 50 mg/l kanamycin at 37° C. Plasmid DNA was purified by using Qiaquick Mini  
5 Prep kits (Qiagen, CA).

**EXAMPLE III. TRANSFORMATION OF AGROBACTERIUM WITH THE EXPRESSION VECTOR**

- After the plasmid vector containing the gene was constructed, the vector was used to transform *Agrobacterium tumefaciens* cells expressing the gene products. The stock of  
10 *Agrobacterium tumefaciens* cells for transformation were made as described by Nagel et al. (1990) *FEMS Microbiol Letts.* 67: 325-328. *Agrobacterium* strain ABI was grown in 250 ml LB medium (Sigma) overnight at 28°C with shaking until an absorbance ( $A_{600}$ ) of 0.5 – 1.0 was reached. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4° C. Cells were then resuspended in 250 µl chilled buffer (1 mM HEPES, pH adjusted to 7.0 with KOH). Cells were  
15 centrifuged again as described above and resuspended in 125 µl chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100 µl and 750 µl, respectively. Resuspended cells were then distributed into 40 µl aliquots, quickly frozen in liquid nitrogen, and stored at -80° C.

- Agrobacterium* cells were transformed with plasmids prepared as described  
20 above following the protocol described by Nagel et al. For each DNA construct to be transformed, 50 – 100 ng DNA (generally resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was mixed with 40 µl of *Agrobacterium* cells. The DNA/cell mixture was then transferred to a chilled cuvette with a 2mm electrode gap and subject to a 2.5 kV charge dissipated at 25 µF and 200 µF using a Gene Pulser II apparatus (Bio-Rad). After electroporation, cells were  
25 immediately resuspended in 1.0 ml LB and allowed to recover without antibiotic selection for 2 – 4 hours at 28° C in a shaking incubator. After recovery, cells were plated onto selective medium of LB broth containing 100 µg/ml spectinomycin (Sigma) and incubated for 24-48 hours at 28° C. Single colonies were then picked and inoculated in fresh medium. The presence of the plasmid construct was verified by PCR amplification and sequence analysis.

- 30 **EXAMPLE IV. TRANSFORMATION OF ARABIDOPSIS PLANTS WITH AGROBACTERIUM TUMEFACIENS WITH EXPRESSION VECTOR**

After transformation of *Agrobacterium tumefaciens* with plasmid vectors containing the gene, single *Agrobacterium* colonies were identified, propagated, and used to

transform *Arabidopsis* plants. Briefly, 500 ml cultures of LB medium containing 50 mg/l kanamycin were inoculated with the colonies and grown at 28°C with shaking for 2 days until an absorbance ( $A_{600}$ ) of > 2.0 is reached. Cells were then harvested by centrifugation at 4,000 x g for 10 min, and resuspended in infiltration medium (1/2 X Murashige and Skoog salts (Sigma), 1 X Gamborg's B-5 vitamins (Sigma), 5.0% (w/v) sucrose (Sigma), 0.044  $\mu$ M benzylamino purine (Sigma), 200  $\mu$ l/L Silwet L-77 (Lehle Seeds) until an absorbance ( $A_{600}$ ) of 0.8 was reached.

Prior to transformation, *Arabidopsis thaliana* seeds (ecotype Columbia) were sown at a density of ~10 plants per 4" pot onto Pro-Mix BX potting medium (Hummert International) covered with fiberglass mesh (18 mm X 16 mm). Plants were grown under continuous illumination (50-75  $\mu$ E/m<sup>2</sup>/sec) at 22-23°C with 65-70% relative humidity. After about 4 weeks, primary inflorescence stems (bolts) are cut off to encourage growth of multiple secondary bolts. After flowering of the mature secondary bolts, plants were prepared for transformation by removal of all siliques and opened flowers.

The pots were then immersed upside down in the mixture of *Agrobacterium* infiltration medium as described above for 30 sec, and placed on their sides to allow draining into a 1' x 2' flat surface covered with plastic wrap. After 24 h, the plastic wrap was removed and pots are turned upright. The immersion procedure was repeated one week later, for a total of two immersions per pot. Seeds were then collected from each transformation pot and analyzed following the protocol described below.

#### 20 EXAMPLE V. IDENTIFICATION OF ARABIDOPSIS PRIMARY TRANSFORMANTS

Seeds collected from the transformation pots were sterilized essentially as follows. Seeds were dispersed into in a solution containing 0.1% (v/v) Triton X-100 (Sigma) and sterile H<sub>2</sub>O and washed by shaking the suspension for 20 min. The wash solution was then drained and replaced with fresh wash solution to wash the seeds for 20 min with shaking. After removal of the second wash solution, a solution containing 0.1% (v/v) Triton X-100 and 70% ethanol (Equistar) was added to the seeds and the suspension was shaken for 5 min. After removal of the ethanol/detergent solution, a solution containing 0.1% (v/v) Triton X-100 and 30% (v/v) bleach (Clorox) was added to the seeds, and the suspension was shaken for 10 min. After removal of the bleach/detergent solution, seeds were then washed five times in sterile distilled H<sub>2</sub>O. The seeds were stored in the last wash water at 4°C for 2 days in the dark before being plated onto antibiotic selection medium (1 X Murashige and Skoog salts (pH adjusted to 5.7 with 1M KOH), 1 X Gamborg's B-5 vitamins, 0.9% phytagar (Life Technologies), and 50 mg/l kanamycin). Seeds were germinated under continuous illumination (50-75  $\mu$ E/m<sup>2</sup>/sec) at 22-23°C

C. After 7-10 days of growth under these conditions, kanamycin resistant primary transformants (T<sub>1</sub> generation) were visible and obtained. These seedlings were transferred first to fresh selection plates where the seedlings continued to grow for 3-5 more days, and then to soil (Pro-Mix BX potting medium).

- 5                    Primary transformants were crossed and progeny seeds (T<sub>2</sub>) collected; kanamycin resistant seedlings were selected and analyzed. The expression levels of the recombinant polynucleotides in the transformants varies from about a 5% expression level increase to a least a 100% expression level increase. Similar observations are made with respect to polypeptide level expression.

10

#### EXAMPLE VI. IDENTIFICATION OF ARABIDOPSIS PLANTS WITH TRANSCRIPTION FACTOR GENE KNOCKOUTS

- The screening of insertion mutagenized *Arabidopsis* collections for null mutants in a known target gene was essentially as described in Krysan et al (1999) Plant Cell 11:2283-2290. Briefly, gene-specific primers, nested by 5-250 pb to each others, were designed from the 5' and 3' regions of a known target gene. Similarly, nested sets of primers were also created specific to each of the T-DNA or transposon ends (the "right" and "left" borders). All possible combinations of gene specific and T-DNA/transposon primers were used to detect by PCR an insertion event within or close to the target gene. The amplified DNA fragments were then sequenced which allows the precise determination of the T-DNA/transposon insertion point relative to the target gene. Insertion events within the coding or intervening sequence of the genes were deconvoluted from a pool comprising a plurality of insertion events to a single unique mutant plant for functional characterization. The method is described in more detail in Yu and Adam, US Application Serial No. 09/177,733 filed October 23, 1998.

25                    EXAMPLE VII. IDENTIFICATION OF STRUCTURE AND DEVELOPMENT CHARACTERISTICS PHENOTYPE IN OVEREXPRESSOR OR GENE KNOCKOUT PLANTS

- Experiments were performed to identify those transformants or knockouts that exhibited a modified structure and development characteristics. For such studies, the transformants were observed by eye to identify novel structural or developmental characteristics associated with the ectopic expression of the polynucleotides or polypeptides of the invention.

30                    Table 3 shows the phenotypes observed for particular overexpressor or knockout plants and provides the SEQ ID No., the internal reference code (GID), whether a knockout or overexpressor plant was analyzed and the observed phenotype.

**Table 3**

SEQ ID No.	GID	Knockout (KO) or overexpressor (KO)	Phenotype observed
1	G727	OE	Plants were small, and more dark green in color, late flowering and poorly fertile.
3	G732	OE	Plants were small and inflorescence was unelongated. Flowers parts appeared to be unelongated and the plants were semi-sterile.
5	G9	OE	Increased root mass
7	G428	OE	Lobed and highly serrated leaves and abnormal first and second whorl floral organs
9	G869	OE	Undeveloped or small anthers
11	G1269	OE	Extended petioles and leaves pointed upwards
13	G1038	OE	Altered leaf shape
15	G438	KO	Reduced lignin in stem
17	G571	KO	Delayed senescence at the end of the plant lifecycle
19	G748	OE	More vascular bundles in stem
21	G431	OE	Severe developmental abnormalities such as altered branching, twisted rosette leaves, flowers with missing pistils, fused stamens and atypical numbers of petals and stamens, reduced secondary bolts, and lack of cauline leaves.
23	G187	OE	Plants had long, thin cotyledons and reduced apical dominance. Several flower abnormalities, including underdeveloped, sepaloid petals and underdeveloped anthers were also observed.
25	G470	OE	Plants were sterile due to failure of anthers to elongate
27	G615	OE	Plants were sterile due to failure of anthers to develop and failure of stamens to elongate. Fused cotyledons and absence of a shoot apical meristem and true leaves was also observed.
29	G1073	OE	Increased plant size and serrated leaves

- For a particular overexpressor that shows a less beneficial structure and development characteristic, it may be more useful to select a plant with a decreased expression of the particular transcription factor. For a particular knockout that shows a less beneficial structure and development characteristic, it may be more useful to select a plant with an increased expression of the particular transcription factor.

**EXAMPLE VIII. IDENTIFICATION OF HOMOLOGOUS SEQUENCES**

Homologous sequences from *Arabidopsis* and plant species other than *Arabidopsis* were identified using database sequence search tools, such as the Basic Local Alignment Search Tool (BLAST) (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410; and Altschul et al. (1997) *Nucl. Acid Res.* 25: 3389-3402). The tblastx sequence analysis programs were employed using the BLOSUM-62 scoring matrix (Henikoff, S. and Henikoff, J. G. (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919).

Identified *Arabidopsis* homologous sequences are provided in Figure 2 and included in the Sequence Listing. The percent sequence identity among these sequences is as low as 47% sequence identity. Additionally, the entire NCBI GenBank database was filtered for sequences from all plants except *Arabidopsis thaliana* by selecting all entries in the NCBI GenBank database associated with NCBI taxonomic ID 33090 (Viridiplantae; all plants) and excluding entries associated with taxonomic ID 3701 (*Arabidopsis thaliana*). These sequences were compared to sequences representing genes of SEQ IDs Nos. 1-54 on 9/26/2000 using the Washington University TBLASTX algorithm (version 2.0a19MP). For each gene of SEQ IDs Nos. 1-54, individual comparisons were ordered by probability score (P-value), where the score reflects the probability that a particular alignment occurred by chance. For example, a score of  $3.6e-40$  is  $3.6 \times 10^{-40}$ . For up to ten species, the gene with the lowest P-value (and therefore the most likely homolog) is listed in Figure 3.

In addition to P-values, comparisons were also scored by percentage identity. Percentage identity reflects the degree to which two segments of DNA or protein are identical over a particular length. The ranges of percent identity between the non-*Arabidopsis* genes shown in Figure 3 and the *Arabidopsis* genes in the sequence listing are: SEQ ID No. 1: 36%-69%; SEQ ID No. 3: 46%-54%; SEQ ID No. 5: 57%-72%; SEQ ID No. 7: 54%-69%; SEQ ID No. 9: 31%-68%; SEQ ID No. 11: 47%-90%; SEQ ID No. 13: 34%-82%; SEQ ID No. 15: 49%-88%; SEQ ID No. 17: 56%-67%; SEQ ID No. 19: 39%-61%; SEQ ID No. 21: 61%-87%; SEQ ID No. 23: 38%-85%; SEQ ID No. 25: 44%-94%; SEQ ID No. 27: 35%-44%; SEQ ID No. 29: 37%-71%; SEQ ID No. 31: 38%-77%; SEQ ID No. 33: 57%-69%; SEQ ID No. 35: 54%-69%; SEQ ID No. 37: 60%-75%; SEQ ID No. 39: 47%-65%; SEQ ID No. 41: 60%-88%; SEQ ID No. 43: 43%-87%; and SEQ ID No. 45: 53%-97%.

The polynucleotides and polypeptides in the Sequence Listing and the identified homologous sequences may be stored in a computer system and have associated or linked with the sequences a function, such as that the polynucleotides and polypeptides are useful for modifying the structure and development characteristics of a plant.



All references, publications, patents and other documents herein are incorporated by reference in their entirety for all purposes. Although the invention has been described with reference to the embodiments and examples above, it should be understood that various  
5 modifications can be made without departing from the spirit of the invention.

What is claimed is:

1. A transgenic plant with modified structure and development characteristics, which plant comprises a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:
  - 5 (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-23, or a complementary nucleotide sequence thereof;
  - (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
  - (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-10 1, where N=1-23, or a complementary nucleotide sequence thereof;
  - (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
  - (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
  - (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of 15 any of (a)-(e);
  - (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide that modifies a plant's structure and development characteristics;
  - (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence 20 of any of (a)-(g);
  - (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
  - (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23;
  - 25 (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23; and
  - (l) a nucleotide sequence which encodes a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-23.
- 30 2. The transgenic plant of claim 1, further comprising a constitutive, inducible, or tissue-active promoter operably linked to said nucleotide sequence.
3. The transgenic plant of claim 1, wherein the plant is selected from the group consisting of: soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf,

banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, and vegetable brassicas.

5

4. An isolated or recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-23, or a complementary nucleotide sequence thereof;
- 10 (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
- (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-23, or a complementary nucleotide sequence thereof;
- (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
- 15 (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
- (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
- (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which  
20 subsequence or fragment encodes a polypeptide that modifies a plant's structure and development characteristics;
- (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g);
- (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide  
25 sequence of any of (a)-(g);
- (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23;
- (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-23; and
- 30 (l) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-23.

5. The isolated or recombinant polynucleotide of claim 4, further comprising a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence.
6. A cloning or expression vector comprising the isolated or recombinant polynucleotide of claim 4.
7. A cell comprising the cloning or expression vector of claim 6.
8. A transgenic plant comprising the isolated or recombinant polynucleotide of claim 4.
9. A composition produced by one or more of:
  - (a) incubating one or more polynucleotide of claim 4 with a nuclease;
  - (b) incubating one or more polynucleotide of claim 4 with a restriction enzyme;
  - (c) incubating one or more polynucleotide of claim 4 with a polymerase;
  - (d) incubating one or more polynucleotide of claim 4 with a polymerase and a primer;
  - (e) incubating one or more polynucleotide of claim 4 with a cloning vector, or
  - (f) incubating one or more polynucleotide of claim 4 with a cell.
10. A composition comprising two or more different polynucleotides of claim 4.
11. An isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide of claim 4.
12. A plant ectopically expressing an isolated polypeptide of claim 11.
13. A method for producing a plant having a modified structure and development characteristic, the method comprising altering the expression of the isolated or recombinant polynucleotide of claim 4 or the expression levels or activity of a polypeptide of claim 11 in a plant, thereby producing a modified plant, and selecting the modified plant for modified structure and development characteristics thereby providing the modified plant with a modified structure and development characteristics.
14. The method of claim 13, wherein the polynucleotide is a polynucleotide of claim 4.

15. A method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of claim 4, the method comprising:

- (a) expressing a polypeptide encoded by the polynucleotide in a plant; and
- (b) identifying at least one factor that is modulated by or interacts with the polypeptide.

5

16. The method of claim 15, wherein the identifying is performed by detecting binding by the polypeptide to a promoter sequence, or detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system.

10 17. The method of claim 15, wherein the identifying is performed by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

18. A method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest, the method comprising:

15 (a) placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of claim 4; and,

(b) monitoring one or more of:

- (i) expression level of the polynucleotide in the plant;
- (ii) expression level of the polypeptide in the plant;
- 20 (iii) modulation of an activity of the polypeptide in the plant; or
- (iv) modulation of an activity of the polynucleotide in the plant.

19. An integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of claim 4, or to a polypeptide encoded by the polynucleotide.

25

20. The integrated system, computer or computer readable medium of claim 19, further comprising a link between said one or more sequence strings to a modified plant structure and development characteristics phenotype.

30

21. A method of identifying a sequence similar or homologous to one or more polynucleotides of claim 4, or one or more polypeptides encoded by the polynucleotides, the method comprising:

- (a) providing a sequence database; and,

(b) querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

5

22. The method of claim 21, wherein the querying comprises aligning one or more of the target sequences with one or more of the one or more sequence members in the sequence database.

10

23. The method of claim 21, wherein the querying comprises identifying one or more of the one or more sequence members of the database that meet a user-selected identity criteria with one or more of the target sequences.

15

24. The method of claim 21, further comprising linking the one or more of the polynucleotides of claim 4, or encoded polypeptides, to a modified plant structure and development characteristics phenotype.

20

25. A plant comprising altered expression levels of an isolated or recombinant polynucleotide of claim 4.

26. A plant comprising altered expression levels or the activity of an isolated or recombinant polypeptide of claim 11.

25

27. A plant lacking a nucleotide sequence encoding a polypeptide of claim 11.

Figure 1

SEQ ID No.	GID	cDNA or protein	conserved domain
1	G727	cDNA	
2	G727	protein	226-269
3	G732	cDNA	
4	G732	protein	31-9
5	G9	cDNA	
6	G9	protein	62-127
7	G428	cDNA	
8	G428	protein	229-292
9	G869	cDNA	
10	G869	protein	109-177
11	G1269	cDNA	
12	G1269	protein	27-83
13	G1038	cDNA	
14	G1038	protein	198-247
15	G438	cDNA	
16	G438	protein	22-85
17	G571	cDNA	
18	G571	protein	160-220
19	G748	cDNA	
20	G748	protein	112-140
21	G431	cDNA	
22	G431	protein	286-335
23	G187	cDNA	
24	G187	protein	172-228
25	G470	cDNA	
26	G470	protein	61-393
27	G615	cDNA	
28	G615	protein	88-147
29	G1073	cDNA	
30	G1073	protein	33-42, 78-175

Figure 2

SEQ ID No.	GID	homolog	cDNA or protein	conserved domain
31	G1493	homolog of G727	cDNA	
32	G1493	homolog of G727	protein	242-289
33	G993	homolog of G9	cDNA	
34	G993	homolog of G9	protein	69-134
35	G867	homolog of G9	cDNA	
36	G867	homolog of G9	protein	59-124
37	G1930	homolog of G9	cDNA	
38	G1930	homolog of G9	protein	59-124
39	G1594	homolog of G428	cDNA	
40	G1594	homolog of G428	protein	262-325
41	G391	homolog of G438	cDNA	
42	G391	homolog of G438	protein	25-85
43	G390	homolog of G438	cDNA	
44	G390	homolog of G438	protein	18-81
45	G1548	homolog of G438	cDNA	
46	G1548	homolog of G438	protein	17-77



Figure 3A

SEQ ID No.	GID	Genbank NID	P-value	Species
1	G727	7283684	2.20E-56	Glycine max
1	G727	7206180	8.40E-42	Medicago truncatula
1	G727	7614196	2.20E-40	Lotus japonicus
1	G727	572293	1.20E-31	Oryza sativa
1	G727	7218448	7.70E-30	Sorghum bicolor
1	G727	9291284	1.80E-27	Lycopersicon hirsutum
1	G727	8901641	5.10E-27	Hordeum vulgare
1	G727	8380453	6.60E-24	Gossypium arboreum
1	G727	9962201	2.10E-12	Cryptomeria japonica
1	G727	8122498	3.10E-08	Lycopersicon esculentum
3	G732	5048074	5.60E-30	Gossypium hirsutum
3	G732	4384142	6.10E-30	Lycopersicon esculentum
3	G732	7623218	6.10E-30	Gossypium arboreum
3	G732	4457220	1.80E-29	Capsicum chinense
3	G732	7284989	4.50E-28	Glycine max
3	G732	9650827	1.20E-27	Petroselinum crispum
3	G732	7205618	2.20E-26	Medicago truncatula
3	G732	3854258	1.40E-22	Populus tremula x Populus tremuloides
5	G9	7643366	6.80E-56	Medicago truncatula
5	G9	8669779	4.20E-50	Glycine max
5	G9	8329389	1.50E-48	Mesembryanthemum crystallinum
5	G9	9851335	3.50E-42	Sorghum bicolor
5	G9	7412012	1.50E-41	Lycopersicon esculentum
5	G9	10450225	1.30E-38	Solanum tuberosum
5	G9	8902194	8.30E-36	Hordeum vulgare
5	G9	7722547	2.60E-33	Lotus japonicus
5	G9	9696857	1.90E-32	Triticum aestivum
5	G9	7324245	2.40E-32	Lycopersicon pennellii
7	G428	3327268	5.50E-65	Ipomoea nil
7	G428	4589883	1.20E-60	Nicotiana tabacum
7	G428	1814233	2.20E-56	Solanum tuberosum
7	G428	7581978	8.50E-56	Dendrobium grex Madame Thong-In
7	G428	4098241	1.50E-53	Lycopersicon esculentum
7	G428	4099825	1.30E-38	Picea mariana
7	G428	3462611	2.50E-38	Pisum sativum
7	G428	3928842	1.90E-37	Picea abies
7	G428	9699343	2.70E-35	Triticum aestivum
7	G428	1008878	4.80E-35	Zea mays
9	G869	10235055	1.00E-19	Glycine max
9	G869	2213784	1.60E-19	Lycopersicon esculentum
9	G869	3065894	9.20E-19	Nicotiana tabacum
9	G869	8570080	5.30E-18	Oryza sativa
9	G869	7560260	1.90E-17	Medicago truncatula
9	G869	9850452	9.30E-16	Sorghum bicolor
9	G869	9963144	1.10E-13	Cryptomeria japonica
9	G869	9660634	1.90E-13	Secale cereale
9	G869	9362061	3.40E-13	Triticum aestivum
9	G869	7788764	7.20E-13	Lotus japonicus
11	G1269	9565366	7.00E-37	Glycine max
11	G1269	5272360	8.10E-37	Lycopersicon esculentum
11	G1269	9119112	8.40E-28	Medicago truncatula
11	G1269	9852711	2.10E-22	Sorghum bicolor

Figure 3B

SEQ ID No.	GID	Genbank NID	P-value	Species
11	G1269	9255178	1.10E-18	Zea mays
11	G1269	10447957	8.60E-15	Solanum tuberosum
11	G1269	9435251	1.20E-09	Hordeum vulgare
11	G1269	3858030	3.20E-09	Populus balsamifera subsp. trichocarpa
11	G1269	9696112	3.80E-09	Triticum aestivum
11	G1269	8213273	4.90E-09	Oryza sativa
13	G1038	8748344	8.00E-37	Medicago truncatula
13	G1038	7283684	5.20E-36	Glycine max
13	G1038	7218448	8.80E-36	Sorghum bicolor
13	G1038	572293	3.30E-35	Oryza sativa
13	G1038	8901641	4.30E-28	Hordeum vulgare
13	G1038	9962201	2.20E-16	Cryptomeria japonica
13	G1038	7614196	6.50E-11	Lotus japonicus
13	G1038	9291272	0.00015	Lycopersicon hirsutum
13	G1038	8122498	0.0005	Lycopersicon esculentum
13	G1038	9883662	0.68	Triticum aestivum
15	G438	7209474	8.70E-204	Oryza sativa
15	G438	7209911	2.20E-142	Physcomitrella patens
15	G438	7571387	2.30E-80	Medicago truncatula
15	G438	8330425	3.00E-66	Mesembryanthemum crystallinum
15	G438	6531152	1.60E-64	Lycopersicon esculentum
15	G438	6726825	4.70E-61	Glycine max
15	G438	5269007	7.00E-54	Zea mays
15	G438	9253000	1.70E-47	Solanum tuberosum
15	G438	8967371	4.40E-46	Hordeum vulgare
15	G438	2963336	1.60E-34	Pinus taeda
17	G571	6288681	1.50E-70	Nicotiana tabacum
17	G571	297019	1.60E-68	Zea mays
17	G571	10423526	2.20E-61	Oryza sativa
17	G571	5926681	4.20E-61	Triticum aestivum
17	G571	4959969	1.90E-59	Lycopersicon esculentum
17	G571	1372965	1.20E-56	Vicia faba
17	G571	8098832	1.20E-46	Hordeum vulgare
17	G571	9566058	2.00E-43	Glycine max
17	G571	765198	1.50E-41	Solanum tuberosum
17	G571	19679	3.80E-41	Nicotiana sp.
19	G748	853689	7.00E-87	Cucurbita maxima
19	G748	7242897	3.90E-59	Oryza sativa
19	G748	5888560	1.20E-45	Lycopersicon esculentum
19	G748	6341666	5.60E-38	Glycine max
19	G748	10700058	1.10E-36	Medicago truncatula
19	G748	7535776	5.00E-33	Sorghum bicolor
19	G748	9419494	2.10E-31	Hordeum vulgare
19	G748	9410157	1.00E-28	Triticum aestivum
19	G748	3929324	4.30E-25	Dendrobium grex Madame Thong-IN
19	G748	10449922	2.30E-23	Solanum tuberosum
21	G431	7340349	9.90E-177	Brassica oleracea
21	G431	3462611	1.20E-112	Pisum sativum
21	G431	310568	1.50E-112	Glycine max
21	G431	2251078	1.90E-107	Nicotiana tabacum
21	G431	4098239	1.20E-104	Lycopersicon esculentum
21	G431	1008878	4.90E-62	Zea mays
21	G431	6942299	7.90E-62	Triticum aestivum

Figure 3C

SEQ ID No.	GID	Genbank NID	P-value	Species
21	G431	3327239	1.90E-61	Oryza sativa
21	G431	3928842	1.60E-59	Picea abies
21	G431	2522483	2.30E-59	Hordeum vulgare
23	G187	9304207	2.10E-35	Sorghum bicolor
23	G187	9444636	3.20E-34	Triticum aestivum
23	G187	5058292	3.60E-34	Glycine max
23	G187	7721184	2.40E-32	Lotus japonicus
23	G187	7562279	1.20E-31	Medicago truncatula
23	G187	8105974	3.00E-29	Lycopersicon esculentum
23	G187	9049477	1.60E-27	Oryza sativa
23	G187	9187621	1.60E-23	Solanum tuberosum
23	G187	5268376	5.60E-23	Zea mays
23	G187	4894964	1.70E-22	Avena sativa
25	G470	6917173	4.80E-78	Lycopersicon pennellii
25	G470	8827792	8.50E-70	Glycine max
25	G470	5272309	7.40E-69	Lycopersicon esculentum
25	G470	7563870	6.70E-68	Medicago truncatula
25	G470	5296108	5.50E-65	Zea mays
25	G470	7339690	7.40E-57	Oryza sativa
25	G470	5047367	1.30E-51	Gossypium hirsutum
25	G470	9856054	9.70E-50	Sorghum bicolor
25	G470	3857884	1.10E-38	Populus balsamifera subsp. trichocarpa
25	G470	8174666	6.40E-37	Hordeum vulgare
27	G615	5566284	2.00E-28	Linaria vulgaris
27	G615	6358617	3.20E-27	Antirrhinum graniticum
27	G615	6358613	1.40E-26	Antirrhinum majus subsp. cirrhigerum
27	G615	6358545	8.60E-26	Digitalis purpurea
27	G615	6358538	1.40E-25	Antirrhinum braun-blauquetii
27	G615	6358541	1.40E-25	Misopates orontium
27	G615	6358542	1.40E-25	Antirrhinum molle
27	G615	6358573	1.40E-25	Misopates calycinum
27	G615	6358546	1.80E-25	Antirrhinum siculum
27	G615	2826867	2.70E-25	Antirrhinum majus
29	G1073	7238733	2.70E-55	Medicago truncatula
29	G1073	10843924	1.50E-44	Glycine max
29	G1073	7615218	2.00E-42	Lotus japonicus
29	G1073	7333102	3.40E-34	Lycopersicon esculentum
29	G1073	9689692	8.60E-28	Pinus taeda
29	G1073	9445090	4.30E-25	Triticum aestivum
29	G1073	9252370	2.80E-24	Solanum tuberosum
29	G1073	5042437	5.80E-21	Oryza sativa
29	G1073	7536402	6.70E-20	Sorghum bicolor
29	G1073	9662742	2.70E-19	Secale cereale
31	G1493	7614196	2.20E-50	Lotus japonicus
31	G1493	9986889	6.10E-48	Glycine max
31	G1493	8748344	2.20E-38	Medicago truncatula
31	G1493	572293	1.70E-37	Oryza sativa
31	G1493	7218448	5.70E-33	Sorghum bicolor
31	G1493	9291284	9.70E-32	Lycopersicon hirsutum
31	G1493	8380453	1.60E-30	Gossypium arboreum
31	G1493	8901641	1.70E-30	Hordeum vulgare
31	G1493	9962201	6.90E-17	Cryptomeria japonica
31	G1493	8122498	1.50E-08	Lycopersicon esculentum

Figure 3D

SEQ ID No.	GID	Genbank NID	P-value	Species
33	G993	7643366	1.20E-58	Medicago truncatula
33	G993	8329389	1.00E-49	Mesembryanthemum crystallinum
33	G993	8669779	6.10E-49	Glycine max
33	G993	9851335	6.30E-43	Sorghum bicolor
33	G993	4384549	5.20E-40	Lycopersicon esculentum
33	G993	10450225	3.70E-39	Solanum tuberosum
33	G993	8902194	2.50E-34	Hordeum vulgare
33	G993	7719409	1.30E-32	Lotus japonicus
33	G993	8749037	5.20E-32	Citrus x paradisi
33	G993	9247126	1.30E-30	Oryza sativa
35	G867	7643366	2.20E-57	Medicago truncatula
35	G867	8329389	1.10E-50	Mesembryanthemum crystallinum
35	G867	8669779	2.70E-46	Glycine max
35	G867	10450225	3.60E-41	Solanum tuberosum
35	G867	9851335	2.80E-40	Sorghum bicolor
35	G867	9430646	7.20E-40	Lycopersicon esculentum
35	G867	8902194	1.60E-34	Hordeum vulgare
35	G867	7722547	1.30E-33	Lotus japonicus
35	G867	7324245	3.90E-32	Lycopersicon pennellii
35	G867	8749037	1.40E-31	Citrus x paradisi
37	G1930	7643366	9.70E-57	Medicago truncatula
37	G1930	8329389	4.50E-47	Mesembryanthemum crystallinum
37	G1930	6069592	1.10E-46	Glycine max
37	G1930	10450225	6.50E-42	Solanum tuberosum
37	G1930	9430646	8.20E-39	Lycopersicon esculentum
37	G1930	9851335	1.80E-38	Sorghum bicolor
37	G1930	7722547	4.70E-34	Lotus japonicus
37	G1930	7324245	1.20E-32	Lycopersicon pennellii
37	G1930	8902194	3.00E-31	Hordeum vulgare
37	G1930	9697984	4.60E-29	Triticum aestivum
39	G1594	3327268	2.60E-74	Ipomoea nil
39	G1594	7581978	9.20E-62	Dendrobium grex Madame Thong-In
39	G1594	4887609	1.50E-47	Oryza sativa
39	G1594	1814233	4.00E-46	Solanum tuberosum
39	G1594	4589883	6.30E-43	Nicotiana tabacum
39	G1594	4098241	6.70E-43	Lycopersicon esculentum
39	G1594	3928842	2.00E-42	Picea abies
39	G1594	4099825	2.60E-42	Picea mariana
39	G1594	4240538	1.70E-41	Zea mays
39	G1594	1946219	1.90E-41	Malus domestica
41	G391	7209474	4.70E-194	Oryza sativa
41	G391	7209911	2.10E-145	Physcomitrella patens
41	G391	7560927	8.70E-67	Medicago truncatula
41	G391	10808354	1.50E-61	Solanum tuberosum
41	G391	5893826	7.00E-60	Lycopersicon esculentum
41	G391	8330425	8.60E-59	Mesembryanthemum crystallinum
41	G391	8284059	8.70E-57	Glycine max
41	G391	5269007	8.10E-46	Zea mays
41	G391	9419425	1.70E-43	Hordeum vulgare
41	G391	2963336	2.10E-37	Pinus taeda
43	G390	7209474	2.50E-166	Oryza sativa
43	G390	7209911	1.70E-149	Physcomitrella patens
43	G390	7560927	5.80E-81	Medicago truncatula

Figure 3E

SEQ ID No.	GID	Genbank NID	P-value	Species
43	G390	7409018	1.80E-68	Lycopersicon esculentum
43	G390	8071613	3.00E-60	Solanum tuberosum
43	G390	9466042	1.60E-59	Hordeum vulgare
43	G390	8284059	1.00E-57	Glycine max
43	G390	8330425	2.60E-44	Mesembryanthemum crystallinum
43	G390	5269007	4.60E-44	Zea mays
43	G390	2963336	4.90E-43	Pinus taeda
45	G1548	7209474	5.90E-169	Oryza sativa
45	G1548	7209911	3.30E-140	Physcomitrella patens
45	G1548	9253000	1.60E-76	Solanum tuberosum
45	G1548	9820423	1.40E-67	Glycine max
45	G1548	7570825	8.40E-67	Medicago truncatula
45	G1548	9456848	2.70E-55	Lycopersicon esculentum
45	G1548	9419425	1.40E-47	Hordeum vulgare
45	G1548	6626571	3.50E-46	Zea mays
45	G1548	8330425	4.20E-46	Mesembryanthemum crystallinum
45	G1548	3853847	2.70E-42	Populus tremula x Populus tremuloides

## MBI0018 Sequence Listing.ST25

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## MBI0018 Sequence Listing.ST25

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## MBI0018 Sequence Listing.ST25

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